

## Null Results in Brief

# Selenomethionine Treatment Does Not Alter Gene Expression in Normal Squamous Esophageal Mucosa in a High-Risk Chinese Population

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## Abstract

Selenium is a promising cancer chemoprevention agent. A recent randomized controlled chemoprevention trial found that selenomethionine (SeMet) supplementation for 10 months favorably effected a change in esophageal dysplasia grade among participants who started the trial with mild dysplasia. To further explore the role of SeMet in this trial, we compared gene expression profiles by treatment group using Affymetrix HU 133A chips in before/after supplementation paired normal esophageal biopsies from a subset of 29 trial participants, 16 who received SeMet, and 13 who

received placebo. Using  $P < 0.001$  as a cutoff, 11 differentially expressed genes were found in the SeMet supplementation group but these genes did not include either known selenoprotein genes or genes previously shown to be modulated by selenium treatment. Because the number of differentially expressed genes ( $n = 11$ ) was less than expected by chance ( $n = 18$ ), we concluded that SeMet supplementation had no measurable effect on gene expression in the normal squamous esophagus of these subjects with dysplasia. (Cancer Epidemiol Biomarkers Prev 2006;15(5):1046–7)

## Introduction

Selenium compounds have been widely studied in the etiology of cancer and as chemoprevention agents because of their potential anticarcinogenic properties (1). Selenium deficiency may play an important role in the etiology of esophageal squamous cell carcinoma in the high-risk population of Linxian, China, where this cancer is endemic and low serum selenium concentrations are strongly associated with increased risk of esophageal squamous cell carcinoma (2). The chemopreventative effects of selenomethionine (SeMet) and celecoxib were recently assessed in a randomized, double-blind, placebo-controlled,  $2 \times 2$  factorial chemoprevention trial conducted in Linxian (3). This trial found that among patients with mild esophageal squamous dysplasia, 10 months of daily treatment with 200  $\mu$ g SeMet favorably effected a change in dysplasia grade, such that there was less progression and more regression in SeMet recipients compared with participants who did not receive SeMet ( $P = 0.02$ ). The precise mechanisms underlying the action of SeMet have not been defined. The present study addressed this gap in knowledge by investigating potential changes in gene expression in normal esophageal mucosa from SeMet- and placebo-treated participants of this trial.

## Materials and Methods

Details of the chemoprevention trial have been described elsewhere (3). Briefly, asymptomatic adults with histologically confirmed mild or moderate esophageal squamous dysplasia were randomly assigned to one of four intervention groups using a  $2 \times 2$  factorial design. Active treatments were SeMet 200  $\mu$ g once per day and/or celecoxib 200 mg twice per day. Esophagogastroduodenoscopy examinations with Lugol's iodine staining were conducted and biopsies were collected and snap frozen before and after a 10-month intervention period.

We compared gene expression in paired histologically normal biopsies from the same individual, one collected at trial baseline ( $T_0$ ) and the second collected at the end of the intervention period ( $T_{10}$ ), from each of 29 subjects, including 16 who received SeMet supplementation and 13 who received placebo. Serum selenium concentrations were also examined from blood samples collected at baseline and at the end of supplementation using Inductively Coupled Plasma Mass Spectroscopy (4). The study was approved by the Institutional Review Boards of the Cancer Institute, Chinese Academy of Medical Sciences and the U.S. National Cancer Institute.

RNA was extracted from frozen biopsies using standard methods. The small sample RNA amplification protocol described in detail elsewhere was used for this analysis (5). Affymetrix HU 133A chip arrays, consisting of 18,400 transcripts, including 14,500 known genes, were scanned in an Affymetrix GCOS Argon-Ion Scanner at 488 nm. Analyses included probe-level preprocessing using robust multiarray analysis (justRMA) conducted with a 64-bit version of R 1.8.1 and Bioconductor 1.3 on the NIH/CIT Helix System and S-Plus 6.1. To control for multiple comparisons, we considered  $P < 0.001$  as statistically significant. All tests were two sided.

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**Table 1. Genes differentially expressed in normal mucosa of the esophagus following SeMet treatment ( $n = 29$  subjects)**

Gene*	UniGene ID	Difference between SeMet and placebo groups from $T_0$ to $T_{10}$ (%) <sup>†</sup>	P
Expression increased			
<i>SOD2</i>	Hs.384944	143	0.00077
<i>MAX</i>	Hs.42712, Hs.497322	130	0.00030
<i>GNAS</i>	Hs.157307	122	0.00054
<i>C5orf3</i>	Hs.166551	116	0.00024
<i>SCL39A8</i>	Hs.284205	112	0.00083
Expression decreased			
<i>LRRN4</i>	Hs.125742	87	0.00091
<i>HIST1H4G</i>	Hs.519634	84	0.00060
<i>SMA3</i>	Hs.440958	80	0.00008
<i>SIAT4B</i>	Hs.270986	77	0.00049
<i>RPS10</i>	Hs.406620, Hs.472444	75	0.00043
<i>TOB2</i>	Hs.4994	70	0.00042

\*These genes were statistically significant ( $P < 0.001$ ) using  $t$  tests with unequal variances.

<sup>†</sup> Average ( $T_{10}$  gene expression/ $T_0$  gene expression) in SeMet group/average ( $T_{10}$  gene expression/ $T_0$  gene expression) in placebo group.

Two-sample  $t$  tests were used to compare the mean of the individual paired  $T_{10}$  minus  $T_0$  differences by treatment group.

## Results

Expression information for 18,400 transcripts was generated using Affymetrix HU 133 chip A microarrays for 29 subjects with paired histologically normal biopsies from the beginning and the end of the intervention period. At an  $\alpha$  level of 0.001, power calculations showed >90% power to detect a  $\geq 2$ -fold difference in the expression of any single gene from baseline to 10 months in individuals taking SeMet compared with those taking placebo.  $T$  tests revealed only 11 differentially expressed genes with  $P < 0.001$  (Table 1). This is fewer than the number of genes expected to be different by chance alone. That is, at  $P = 0.001$  in an array of  $\sim 18,000$  targets, on average, 18 genes would be expected to show a significant difference by chance. The list of 11 differentially expressed genes did not include the genes for the 25 previously identified selenoproteins (Table 2) or genes previously identified as being modulated by selenium treatment (Table 3; refs. 6-13).

Following 10 months of intervention, the serum selenium concentrations increased substantially (from a median of 84  $\mu\text{g/L}$  to a median of 205  $\mu\text{g/L}$ ) in the SeMet-treated individuals ( $n = 16$ ) but very little in the placebo group (from median of 75  $\mu\text{g/L}$  to a median of 94  $\mu\text{g/L}$ ;  $n = 13$ ).

## Discussion

In this analysis, we did not see a difference in gene expression beyond the number of genes likely to be different by chance, despite a substantial increase in serum selenium concentrations and a study size large enough to provide >90% power to detect  $\geq 2$ -fold differences in gene expression. Had we used alternative, more conservative statistical adjustment strategies

**Table 3. Genes known to be modulated by selenium treatment from the literature ( $n = 22$ )**

<i>NF-<math>\kappa</math>B</i>	Phospho-JNK	CDC25A	GADD45	DHFR
<i>AP-1</i>	Cyclin A	P21 <sup>WAF1</sup>	GADD153	Cdk1
<i>Cdk2</i>	Cyclin B1	Cdk5	P27	
<i>Cyclooxygenase</i>	Cyclin D	DP1	Bcl-2	
<i>Lipoxygenase</i>	<i>Cdc2</i>	<i>c-jun</i>	CKH2	

(e.g., Bonferonni or Benjamini-Hochberg), we would have identified no differentially expressed genes. In contrast to our findings, changes in gene expression have previously been reported in the mammary glands of rats treated with methylseleninic acid (10). Differences in species, dose, route of exposure, tissue specificity, and form of selenium may explain this apparent discrepancy in results.

There are several potential explanations for our findings. First, although we documented substantial increases in serum selenium levels, we did not measure selenium in esophageal tissue and selenium might not concentrate there. Second, we studied only histologically normal tissue, not dysplastic lesions. We do not know if gene expression results would be the same in premalignant lesions. Third, laboratory assay limitations might have systematically dampened the differences observed. Supplementation-related increase in serum selenium levels and directly observed therapy indicate that noncompliance was not an explanation.

In conclusion, our results show that 10 months of 200  $\mu\text{g}$  daily SeMet treatment had no measurable effect on gene expression profiles in the normal squamous esophageal mucosa of these high-risk Chinese patients. This lack of statistically significant differences in gene expression is surprising because the chemoprevention trial showed a beneficial effect of SeMet in patients with mild esophageal dysplasia.

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**Table 2. Known selenoproteins ( $n = 25$ )**

GPX1	TR1	DIO3	SELR	SELV
GPX2	TR2	SEPP1	SELK	SELO
GPX3	TR3	SELN	SELH	SELI
GPX4	DIO1	SELW	SELM	SEP15
GPX6	DIO2	SPS2	SELS	SELT